

# Interactions of Platinum Metals and Their Complexes in Biological Systems

by André F. LeRoy\*

Platinum-metal oxidation catalysts are to be introduced in exhaust systems of many 1975 model-year automobiles in the U.S. to meet Clean Air Act standards. Small quantities of finely divided catalyst have been found issuing from prototype systems; platinum and palladium compounds may be found also.

Although platinum exhibits a remarkable resistance to oxidation and chemical attack, it reacts chemically under some conditions producing coordination complex compounds. Palladium reacts more readily than platinum.

Some platinum-metal complexes interact with biological systems as bacteriostatic, bacteriocidal, viricidal, and immunosuppressive agents. Workers chronically exposed to platinum complexes often develop asthma-like respiratory distress and skin reactions called platinosis. Platinum complexes used alone and in combination therapy with other drugs have recently emerged as effective agents in cancer chemotherapy. Understanding toxic and favorable interactions of metal species with living organisms requires basic information on quantities and chemical characteristics of complexes at trace concentrations in biological materials. Some basic chemical kinetic and thermodynamic data are presented to characterize the chemical behavior of the complex  $cis-[Pt(NH_3)_2Cl_2]$  used therapeutically. A brief discussion of quantitation of platinum at nanogram levels in biological tissue is included.

## Introduction

The platinum metals comprise the rare metals in two triads of Group VIII of the periodic classification of elements: ruthenium (Ru), rhodium (Rh), palladium (Pd), and osmium (Os), iridium (Ir), and platinum (Pt). Standards for mobile emission sources (primarily automobiles) have been promulgated; manufacturers have chosen to meet the standards by equipping automobiles with devices using platinum-group catalysts.

Losses of the active metals from these devices will expose large numbers of people to a new environmental contaminant whose biological effects may be important. Interactions of platinum metals and their compounds with biological systems have been reported over the last 140 years. The discovery that platinum metal complexes have a potent effect on cell division in bacteria and some animal tumors prompted more intensive study of their effects on cancers of many types. There is evidence that these platinum-metal complexes interact strongly with some amino acids, peptides, proteins, nucleotides, and nucleosides, specifically with deoxyribonucleic acid (DNA).

This paper summarizes information available on behavior of platinum metals and

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compounds and effects these materials produce in biological systems exposed to them. It describes a method of quantitation of platinum at nanogram levels in biological samples by atomic absorption spectrometry.

## Catalytic Converters

Catalytic converters containing an estimated 1–3 g (approximately 0.1 oz troy), of platinum (Pt) and palladium (Pd) metal have been developed to oxidize unburned hydrocarbon (HC) and carbon monoxide (CO) emissions in internal combustion engine (ICE) exhaust to water ( $H_2O$ ) and carbon dioxide ( $CO_2$ ) to meet the standards established in the Clean Air Act (1). Antiknock additives containing lead alkyls and organic halide scavengers will not be used in the fuels for converter-equipped vehicles because they inactivate the catalysts. Sulfur in the fuel is oxidized to sulfur dioxide ( $SO_2$ ) in the engine; this  $SO_2$  is further oxidized in the converter to the trioxide,  $SO_3$ , that combines with water to produce a mist of sulfuric acid ( $H_2SO_4$ ) in the exhaust gas stream.

Conversion equipment involving use of a ruthenium-based catalyst is under test to diminish oxides of nitrogen ( $NO_x$ ) in the exhaust by chemical reduction to molecular nitrogen ( $N_2$ ) (2).

## Catalyst and Emission Characteristics

The catalysts under consideration may be monolithic or pelletized, with an alumina matrix to support the active metal. Detailed procedures used to manufacture these catalysts are proprietary information that is not generally available.

Industrial vehicles such as ICE-powered fork-lift trucks used in ships' holds, warehouses, and mines have been equipped with catalytic converters for a decade or more. Although vehicle operating conditions and catalyst types may differ, this application seems to offer the best available basis for assessment of these converters in actual use. The U.S. Environmental Protection Agency

has made tests on a Ford Motor Company automotive engine prototype using a monolithic catalyst in its exhaust system and an Oldsmobile (General Motors Corp.) test vehicle equipped with a pelletized catalyst.

Data available on operating parameters (reaction rates, temperature profiles, flow rates and configurations) in catalytic converters of each type under various operating conditions have been closely held by the automakers and catalyst manufacturers and have not been disclosed.

In industrial applications, platinum gauze oxidizing catalysts are known to undergo substantial surface rearrangements during catalysis at temperatures far below the melting point of the metal. Dendritic growths appear, grain sizes increase, and attrition occurs (3). In vehicles, there may be significant differences in the form of catalyst emissions from the different types under test, e.g., alumina particles are found in the exhaust from a vehicle with a pelletized catalyst. Catalytic noble metals in the automobile exhaust gas stream are expected to be very finely divided and predominantly water-insoluble.

Loss rates of platinum and palladium of the order of micrograms of metal per mile have been estimated from regularly serviced test vehicles under running conditions stipulated for the modified California test cycle. Definitive data are lacking on this point. Industry spokesmen expect recovery of the catalytic metals to be economically feasible. Until the recovery rate is established, we note that the automakers have arranged substantial supply contracts. For the coming decade, GM agreed to buy 120,000 troy oz Pd annually and 300,000 troy oz Pt annually from Impala Platinum Ltd. of South Africa. (4).

In addition to their catalytic activity, platinum metals are well known for their resistance to chemical attack, even at elevated temperature. Indeed, many applications of the group are predicated on this inertness. It is less well known that the platinum group metals do react to a degree

that depends on their fineness (or compactness), on the presence and character of impurities, metallurgical history, and conditions of dissolution (5).

There may be a tendency to think the nobility and chemical resistance of the bulk metal extends to the finely divided form and conclude that it is nearly unreactive and must therefore be innocuous. According to Beamish (5), given sufficient fineness, the platinum metals may be expected to respond to the corrosive action of even single mineral acids, particularly in the presence of air. Losses of 1–8 g of platinum per metric ton of sulfuric acid, depending on concentration, were reported for some of the early methods of production of the acid in platinum-lined vessels (6). Palladium is subject to attack by hot concentrated mineral acids.

It is not possible to state, definitively, the extent to which sulfates issuing from a catalytic exhaust system may include those of platinum or palladium, without accurate information on the conditions that obtain in the system.

Platinum is subject to net weight loss when heated strongly in air or oxygen. Depending on conditions, it may incur a loss of weight due to volatility of an oxide or a gain resulting from formation of a stable oxide (5). This point has been treated in an extensive discussion of a century's conflicting reports of reactions under many different conditions (7). Formation of intermetallic alloys within the platinum group or with associated base metals is well known; the effect of such alloying on the resistance to dissolution cannot be simply inferred from the behavior of the components (5).

## Biological Response to Metals and Metal Complexes

The critical question in biological term is: What interactions obtain in living systems exposed to trace concentrations of these highly active metals, whether soluble or insoluble, and to the interconversion products they may form in the environment?

## Microbial Viral and Fungal Interactions

Zinno and Cutolo (8) reported an iridium chloride solution to be highly potent against *Staphylococcus aureus* and the causative organisms of typhoid, cholera, and anthrax. A 10-min exposure to a 0.01N solution rendered spores of the anthrax bacillus non-viable.

Shulman and Dwyer (9) in an extensive review, compared the bacteriostatic behavior of 1,10-phenanthroline and 2,2'-bipyridine bases, their quaternary salts, and their metal chelates containing identical or mixed ligands on gram-positive (*Staphylococcus pyogenes*, *Streptococcus pyogenes*, and *Clostridium welchii*), gram-negative (*Escherichia coli* and *Proteus vulgaris*), and acid-fast (*Mycobacterium tuberculosis* H37Rv) organisms. They found ruthenium complexes moderately active against the gram-positive organisms and showed some activity against the gram-negative microorganisms. They also report that the expected development of drug resistance with *Staph. pyogenes* and *M. tuberculosis* did not occur with the highly active metal chelates of ruthenium and iron. They report further on the inhibitory and mutagenic activity of metal chelates on a yeast, *Saccharomyces cerevisiae*, and fungistatic activity on several pathogenic fungi.

Bromfield et al. (10) found rhodium complexes of the type  $trans-[RhL_2X_2Y]$  (where L is substituted pyridine; X denotes  $Cl^-$  or  $Br^-$ ; Y may be  $Cl^-$ ,  $Br^-$ ,  $NO_3^-$ , or  $ClO_4^-$ ) had high levels of antibacterial activity. Growth of gram-positive organisms was prevented at concentrations of 10–100 ppm. Gram-negative bacteria (except *E. coli*) generally required concentrations 10- to 100-fold higher to arrest growth. Pyridine, substituted pyridines, and rhodium trichloride are themselves ineffective in preventing bacterial growth at 1000 ppm. Thus, the complexes exhibiting the antibacterial activity possess the specific characteristics resulting in this inhibition.

Rosenberg et al. (11) reported cultures of *E. coli* B were killed at part-per-million levels of  $[PtCl_6]^{2-}$  and complexes of rhodium

and ruthenium. Photocatalyzed aquation of the platinum complex resulted in  $[\text{PtCl}_4\text{A}_2]$  (where A is a monodentate ligand, such as an amine) which interferes with the process of cell division and results in filamentous growth.

#### Systemic Administration of Transition Metal Compounds in Mice

Collier and Kraus (12) treated tumorous mice with some 64 transition metal chlorides, including those of three platinum metals. They reported a slight activity on mouse sarcoma by two ruthenium compounds and no significant effect by the rhodium and osmium compounds they used. Taylor and Carmichael (13) studied effects of some 37 transition metal chlorides and nitrates on mouse mammary adenocarcinoma and transplantable sarcoma in DBA mice. The results were favorable with rhodium and iridium chlorides, but apparently these agents were not studied further. Rosenberg (14) also found that the square-planar complexes  $\text{cis-}[\text{PtCl}_2(\text{NH}_3)_2]$ ,  $[\text{Pt}(\text{en})\text{Cl}_2]$ , and the octahedral complexes  $\text{cis-}[\text{Pt}(\text{NH}_3)_2\text{Cl}_4]$  and  $[\text{Pt}(\text{en})\text{Cl}_4]$  (where en denotes ethylenediamine) arrest some tumor growths, e.g., sarcoma-180 in mice, and are effective also against murine leukemia (L-1210) cells. Gale et al. (15) found that photochemical reaction products of ammonium hexachloroiridate (IV),  $(\text{NH}_4)_2[\text{IrCl}_6]$ , retard the rate of development of Ehrlich ascites carcinoma in BALB/c mice and to a lesser degree the development of the L1210 leukemia in BDF<sub>1</sub> mice.

#### Metal Implants in Rats and Mice

Nothdurft (16-18) reported a high frequency of appearance of tumors in rats and mice at the sites of subcutaneously and intraperitoneally implanted discs, spheres, and powderlike forms of the metals platinum, gold, and silver and the nonmetal ivory. He states, categorically, that the appearance of tumors at the site of implantation is not

related to the chemical properties of the metals, but unfortunately he does not adduce results with positive and negative controls to support the conclusion.

#### Human Chronic Exposure to Precious Metals in Industry

Workers chronically exposed to precious metal dusts and their complex salts in platinum metal refineries and in catalyst manufacture are subject to platinosis, a condition whose signs are: cold symptoms, tightness in the chest, dermatitis, eczema and skin ulcerations. Fothergill (19) found none of the symptoms of platinosis in workers exposed to 5-70  $\mu\text{g}/\text{m}^3$  of platinum dust only or to complexes of precious metals other than platinum. Karasek and Karasek (20) identified eight cases of allergic phenomena occurring in the nasal mucosa, bronchi, and skin of photographic studio personnel. They worked with a sensitized paper containing potassium chloroplatinite. Hunter et al. (21) described the same syndrome in 52 of 91 men exposed to dust or spray of complex salts of platinum in four metal refineries in England. It started with repeated sneezing, followed by profuse running of the nose. Tightness of the chest with shortness of breath, wheezing, cough, and cyanosis ensued. Some 13 men also experienced a scaly erythematous dermatitis and some an urticarial rash. These conditions did not occur in workers exposed to much higher concentrations of metallic platinum in the atmosphere or to men exposed to the complex salts of other precious metals including palladium.

Two case histories reported were characteristic: One person worked in the refinery 18 years and had symptoms after the first year which became slowly but progressively worse until he was forced to give up this work. On leaving, the symptoms disappeared and did not recur. The second person presented symptoms after six years of service, becoming worse until asthma appeared in the 10th year. After transfer to another

department, the employee no longer suffered from asthma.

Roberts (22) reviewed the previous reports and cited the experience gained in a five-year study of 21 employees of the Bishop & Co. Platinum Works. He described factors predisposing to platinosis: previous history of allergy, strong family history of hives, hay fever, asthma, contact dermatitis. Individuals who have skin blemishes, moles, acne, sebaceous cysts and other lesions are prone to become sensitive to complex platinum salts. Those with blond hair, blue eyes, and thin or transparent skin that tolerates irritants poorly are prone to have platinosis. More swarthy types, free from skin blemishes with darker, coarse hair and thicker, less sensitive skin do not usually develop the platinum intolerance.

### Biological Effects of Platinum Complexes

Since Rosenberg (14) reported the activity of platinum complexes as antitumor agents in test animals, considerable effort has been made to study the effects in detail. The most widely investigated of the compounds is Peyrone's salt (23-26), *cis*-dichlorodiamminoplatinum(II) (*cis*-[Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>]). It has displayed promising results in arresting tumor growth in animals and has been introduced into clinical trials for cancer treatment in humans to establish appropriate dose levels and schedules.

**Preclinical Toxicology:** Studies of toxicology of *cis*-[Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>] have been conducted on mice and rats (27-30), and have been summarized by Rosenberg (31). The important histological changes observed in these rodents were: denudation of intestinal epithelium, bone marrow depression, thymic and splenic atrophy, and acute nephrosis.

Trials with dogs and monkeys revealed similar toxic effects with damage to the renal tubules a prominent effect, especially at high dose levels. Other important effects are damage to the bone marrow and gastrointestinal epithelium.

**Clinical Experience:** Phase I clinical trials of *cis*-[Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>], administered intravenously, were concluded in mid-1972. In these trials with terminal cancer patients, a number of toxic effects predicted from animal studies were confirmed.

The clinical brochure (32) for *cis*-[Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>] includes the findings of clinicians studying its behavior in humans. Therapeutic efficacy was seen clinically in Phase I trials in treatment of several types of tumors. Some of the most encouraging results were reported by Higby (33) on testicular tumors. The major limitation on the use of this complex as an antitumor agent is its renal toxicity, usually manifested as tubular epithelial damage. It has been estimated that single IV doses above 2 mg/kg leads to unacceptable toxic effects. Other toxic effects reported include ototoxicity, high-frequency hearing loss, at total doses above 1 mg/kg. Wallace (34) has reported no evidence of cumulative renal toxicity in one patient who received 545 mg/m<sup>2</sup> (~14 mg/kg) over a 104-day period (schedule unspecified). Another of his patients received a 479 mg cumulative dose over a period of 200+ days; the patient experienced a BUN elevation of 38 and a creatinine of 4.4 at the very terminal stage of her disease with other complicating factors.

**Mechanism of Action:** Extensive research is under way to establish the mode of antitumor action of this and related complexes. Several interactions with the nucleic acid constituents have been observed and are under study. It has been suggested that tumor destruction results from drug action at sites in the cell resulting in primary lesions on the nuclear DNA (31). The principal mechanism appears to involve inhibition of DNA synthesis and, to a lesser degree, inhibition of RNA and protein synthesis. Harder and Rosenberg (35) reported selective inhibition of DNA synthesis *in vitro* by *cis*-[Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>] below 5 μM.

## Characterization of Platinum Group Metals in Biological Systems

Understanding the detailed interactions of platinum group metals with biological systems requires extensive quantitation and characterization of the chemical species involved. Studies of chemical behavior of complexes in relatively simple systems *in vitro* suggest the large number of variables that affect ligand substitution reactions and thus the diversity of the reaction products.

### Chemical Considerations

Quadrivalent platinum, Pt(IV), and palladium, Pd(IV), form inert, inner orbital ( $d^2sp^3$ ) octahedral complexes.

The most stable square-planar complexes are those of divalent platinum, Pt(II) with  $dsp^2$  hybridization. Divalent palladium, Pd(II), produces complexes of the same geometry; both are low-spin  $d^8$  systems.

Most of the *cis-trans* isomers of square-planar complexes that have been isolated are the relatively inert complexes of Pt(II). In fact, the concept of a square configuration, rather than a tetrahedral one was introduced by Werner (36) because the tetrahedral structure could not account for the two forms of  $[Pt(NH_3)_2Cl_2]$  prepared more than a century ago by Peyrone (23) and by Reiset (37).

### Thermodynamic versus Kinetic Properties of Complexes

The terms "stable" and "unstable" are used to refer to the thermodynamic properties of the complex species considered. The term "inert" is used in the kinetic sense, to describe complexes which engage in ligand replacement reactions slowly; those which undergo such reactions rapidly are described as "labile", as suggested by Taube (38). While it is often true that stable substances are slow to react and unstable compounds react rapidly, there is no absolute requirement that this be the case. Synthesis of various Pt(II) compounds rests on utilization

of competing thermodynamic and kinetic factors. The behavior of the complexes with biological materials will depend on the same considerations; the distinction between thermodynamic and kinetic factors may be important in understanding the interactions. Some metal complexes are so stable they do not react with the biological system. Thus, the stability (stability constant,  $K=10^{44}$ ) and inertness (kinetics extremely slow) of the complex  $[Fe(CN)_6]^{2-}$  are so great, even in acid solution, that it yields no significant HCN; this cyanide complex could be ingested with no ill effects.

Kinetics of the ligand substitution reaction are important, since the half-times of the reactions may range from less than a second to months or years. The latter may appear to be "stable" if only short studies are made. The rates are functions of the geometry of the complex, the metal and its oxidation state, and the polarizability of the ligands. Photochemical effects and pH may also affect reaction rates.

### Effect of Metal Oxidation State

Stability of oxidation states of metal complexes in the biological medium must be considered. Low-spin complexes generally undergo rapid one-electron oxidation or reduction reactions. Since biological systems operate at low redox potential, approximately  $-0.5$  to  $0$  V, reduced low oxidation states usually obtain. Platinum group complexes would be reduced to the metallic state under these conditions but for their inert reduction kinetics. Although Pt(IV) amines would be expected to penetrate biological systems more rapidly than Pt(II) amines, the latter produce the biological effect.

### Photochemical Reactions

Photochemical processes may have an important role in the transformation of platinum complexes: Complexes of the type  $[Pt(NH_3)_nCl_{4-n}]^{n-2}$  undergo photoaquation (39), while *cis*- $[Pt(NH_3)_2(H_2O)_2]^{+2}$  photoisomer-

izes to the *trans* form, and photo-oxidation and reductions have been reported (40,41).

### Common Chemical Features of Antitumor Complexes

Of the transition-metal complexes tested for antitumor activity, only a small number have shown it; some active compounds are highly toxic. The effective compounds have features in common that may aid us in understanding their interactions with biological systems.

Some of these features are outlined here. The complexes exchange some ligands rapidly; other ligands are exchanged slowly or not at all. This behavior is usually found in low-spin complexes having electrons paired in the  $t_{2g}$  level  $d$ -orbitals. Strongly bound ligands are transported with the metal through membranes like cell walls. The geometric configurations of transition metal cations are: square-planar (usually low-spin,  $d^8$ ), pentagonal square-pyramidal (usually low-spin,  $d^7$ ), octahedral (all other low-spin electronic configurations).

Active antitumor agents usually have two exchangeable ligands in *cis* positions. The ligand donor atoms most probable for the low-oxidation state cations in a biological system are N, O, S, Cl, Br; these donor atoms may be incorporated into a large variety of ligands. Some ligands are monodentate; others may be mono- or multi-dentate. Chelation may occur with the latter. Halide ligands can form bridges between metals.

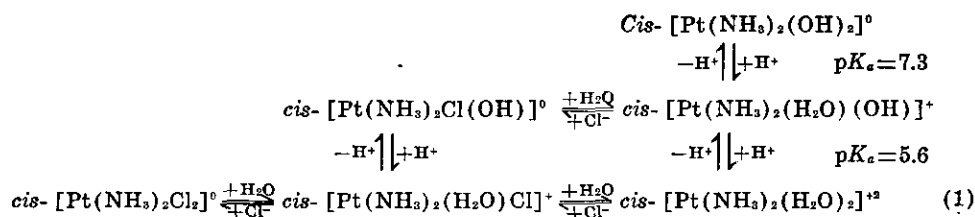
The activity of *cis*-[Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>] against tumors is associated with reactions replacing

the halides. These complexes are bifunctional reagents that may undergo nucleophilic substitution at two *cis* positions.

Antitumor behavior is apparently not related to oxidation-reduction reactions of the metal.

### Detailed Reaction Scheme

Square-planar complexes undergo predominantly bimolecular nucleophilic displacement reactions in contrast to the generally dissociative reactions exhibited by octahedral complexes. *Cis*-[Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>], for example, is injected as a neutral molecule in physiologic saline solution. This neutral species may undergo limited hydrolysis in the extracellular fluid ( $\sim 0.1M$  Cl<sup>-</sup>). Within the cell, a lower chloride ion concentration obtains ( $0.004M$  Cl<sup>-</sup>); thus more extensive hydrolysis may occur as indicated in the stepwise reaction scheme (1), which assumes monomeric species and preservation of the *cis* configuration of the ammine ligands despite the greater favorability of the *trans* configuration thermodynamically. The stability constants shown are cited by Balzini (41). The rate constants for the successive aquation reactions (41) shown for *cis*-[Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>] are  $k_{1,H_2O} = 2.5 \times 10^{-5} \text{ sec}^{-1}$ ,  $k_{2,H_2O} = 3.3 \times 10^{-5} \text{ sec}^{-1}$ ; at 20°C; thus these reactions have half-times of the order of 6–8 hr (42). Note that both ligand substitution rate and partial equilibria for this type of reaction may be functions of the pH and pCl of the surrounding medium. In a fluid low in Cl<sup>-</sup> and, near neutral pH (e.g. cellular fluid), one could expect to find approximately half of the Pt as [Pt(NH<sub>3</sub>)<sub>2</sub>(H<sub>2</sub>O)OH]<sup>+</sup> and half



as  $[\text{Pt}(\text{NH}_3)_2(\text{OH})_2]^0$  if stronger nucleophiles did not displace the hydroxo ligands in the complex molecules.

### Physiochemical Measurements: Properties of Metal Complexes

Electronic absorption spectra yield information on interactions between the metal and bound groups, stability constants and rate constants for complexation. Magnetic and natural circular dichroism (CD) measurements also yield useful information on geometries and metal-ligand interactions. Electron spin resonance (ESR) and nuclear magnetic resonance (NMR) are useful in determining symmetry relations, binding constants and rate data. Vibrational spectra may also be used for characterization of the molecular structures.

A few studies reporting various biological effects reflect known total quantities used in the experiments, but few data are available from analyses for the metal distributed in the biological system. The available data have come principally from tracer experiments with radioactive platinum isotopes.

Renshaw and Thompson (43), performing a tracer study with  $^{191}\text{Pt}$  in *B. cereus*, *S. aureus*, and *E. coli*, showed the bacteriocidal  $[\text{PtCl}_6]^{2-}$  is taken up by the cytoplasmic protein. The photochemical reaction products (principally  $[\text{Pt}(\text{NH}_3)_2\text{Cl}_4]$ ) that produce filamentous growth of the bacteria were bound to nucleic acids and metabolic intermediates, amino acids, peptides, and small nucleic acids as well. Little  $^{191}\text{Pt}$  was bound to the lipid material.

Unfortunately, the platinum radionuclides present experimental difficulties because of the short half-life of the radioactive isotopes and relatively lengthy steps in synthesis and purification of complexes. Studies of long duration require low-level counting facilities if tracer levels introduced initially are to be kept at levels that do not require heroic safety precautions.

### Techniques for Quantitation

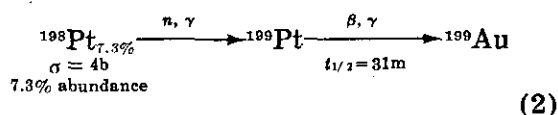
Quantitation of platinum can be performed at relatively low concentrations by several techniques including mass and emission spectrography, arc, spark, and Mössbauer spectroscopy, x-ray fluorescence, neutron activation, and electronic and atomic absorption.

### Standard Reference Materials

The biological Standard Reference Materials (SRM) available from the National Bureau of Standards (NBS) (bovine liver SRM 1577 and mixed orchard leaves SRM 1571) do not contain certified quantities of Pt metals. Laboratories analyzing for these metals have no biological standard to measure their analytical techniques against.

The NBS does offer high purity platinum wire, SRM 680, with a certified analysis from which a hexachloroplatinic acid standard can be prepared. Our determinations by use of atomic absorption spectrophotometry have been based on standards prepared from highly purified Pt metal and a commercially available hexachloroplatinic acid standard.

We plan independent analyses of the platinum in biological materials to confirm results obtained by atomic absorption spectrometry. One method to be used is neutron-activation analysis using the reaction (2):



$^{199}\text{Au}$  subsequently decays to  $^{199}\text{Hg}$  with a 3.15 day half-life.

### Quantitation of Platinum in Biological Samples

Analytical techniques have been evolved to overcome difficulties specific to the types of samples analyzed. Biological samples present special considerations and difficulties; they usually contain a substantial proportion of water, and ionic metal species may bind so strongly to amino acids and proteins that the



latter must be destroyed to free the metal for analysis.

Experience with several procedures for quantitation of platinum in biological samples has led to development of a method that yields reproducible results with tissue samples of approximately 1 g wet weight (or 1 ml samples of fluids like blood plasma or urine) that contain 0.25  $\mu\text{g}$  Pt or more. Measurements typically use a 20- $\mu\text{l}$  portion of digest taken up in 1M HCl. Under favorable conditions, this method yields reliable results with samples containing 2 ng Pt or more.

If analytical results are to be reported on the dry-weight basis, solid tissue is lyophilized. Otherwise, the tissue is weighed wet.

The weighed tissue is wet-ashed in 10 ml of a solution made from 1 volume  $\text{HNO}_3$  (60%) + 1.5 volume  $\text{HClO}_4$  (70%). The digestion step need not be carried out under reflux. The temperature of the mixed acid solution rises progressively as the  $\text{HNO}_3$  is concentrated and volatilized with subsequent concentration of the  $\text{HClO}_4$  and progressive development of its full oxidation potential ( $\sim 2$  V) at 203°C, the boiling point of the  $\text{HClO}_4$ -water azeotrope (acid strength 72.5%). Experience has shown that some tissues (e.g., liver, fat) may require repeated treatment to yield a clear digest. Heating is continued to incipient dryness of the digest, whereupon 5 ml 1M HCl are added and evaporated again to incipient dryness. Finally, the contents of the beaker are taken up into 5 ml 1M HCl.

Portions of this solution (typically, 20  $\mu\text{l}$ ) may be introduced directly into the heated graphite analyzer (HGA-2000) coupled to a Perkin-Elmer 303 atomic absorption spectrophotometer to give consistent results at the 5 ng level or higher by using the 265.9 nm resonance line for Pt.

An additional step using solvent extraction increases the sensitivity of the method. The solvent, methyl isobutyl ketone (MIBK) is used to extract the platinum species from the aqueous phase. This solvent affords a distribution coefficient virtually independent

of HCl concentration in the range 1–12M. The atomic absorption measurement is then made on a 20  $\mu\text{l}$  portion of the solvent. Although this step improves results, we have experienced some unexplained variations in yield with it and consider it premature to recommend.

The solvent extraction technique and variations of it using the aliphatic secondary amines LA-1 or LA-2 liquid ion-exchangers in xylene as extractants are being developed further. We have conducted some preliminary separations using LA-1 since it offers higher distribution coefficients than MIBK over the 1–9M HCl acidity range. Davidson and Jameison (44,45), showed that the extraction depends not only on the HCl molarity in the aqueous phase, but also on the concentration of the background electrolyte anions in the aqueous phase that compete for exchange sites. Work is currently under way to increase the sensitivity of the analysis and improve the reliability of the extraction step.

## Summary

We have cited information on known interactions of platinum-metal complexes with bacteria, viruses and mammals. Effects in humans have been noted for chronic industrial exposure to platinum complex compounds; clinical therapeutic experience with *cis*-[Pt(NH<sub>3</sub>)<sub>2</sub>Cl<sub>2</sub>] has been reported. Knowledge of biological and chemical behavior of platinum metals is limited. Data available on the quantities and chemical nature of the materials issuing from converter equipped test vehicles are insufficient to permit definitive assessment of the risk posed by introduction of these systems on a large scale.

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